Pinto Bean Consumption Changes SCFA Profiles in Fecal Fermentations, Bacterial Populations of the Lower Bowel, and Lipid Profiles in Blood of Humans[1–3]

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Abstract

Beans improve serum lipids and may reduce the risk of colon cancer by increasing colonic SCFA formation. We assessed whether pinto bean consumption affects in vitro fecal bacterial fermentation and production of SCFA, colonic bacterial populations, and serum lipids. Adults grouped as premetabolic syndrome (pre-MetSyn) ($n = 40$) or controls ($n = 40$) were randomly assigned to consume either a bean entrée [1/2 cup (130 g) of dried, cooked pinto beans] or an isocaloric chicken soup entrée daily for 12 wk. Measurements included in vitro fecal fermentation of various resistant starch substrates, fecal bacterial speciation, and blood lipids. When expressed as a difference between baseline and treatment, propionate production from fecal material fermented in vitro with bean flour was higher ($P < 0.02$) in volunteers consuming beans than in those consuming soup. During the treatment period alone, bean consumption did not affect propionic acid production with any substrate but lowered ($P < 0.02$) butyric acid production when cornstarch was the substrate. In all volunteers, bean consumption decreased fecal production of isovaleric ($P < 0.05$) and isobutyric ($P < 0.002$) acids from cornstarch by as much as 50%. Of the bacterial populations tested, only *Eubacterium limosum* was affected by bean consumption and was ~50% lower than in those consuming soup. Beans lowered serum total cholesterol ($P < 0.014$) by ~8% in the controls and 4% in the pre-MetSyn group. Bean consumption lowered serum HDL-cholesterol ($P < 0.05$) and LDL-cholesterol ($P < 0.05$) in both groups without affecting serum triglycerides, VLDL cholesterol, or glucose. This study provides evidence that bean consumption can improve lipid profiles associated with cardiovascular disease, but does not clearly confer health benefits related to colon cancer risk. J. Nutr. 137: 2391–2398, 2007.

Introduction

Cardiovascular disease (CVD)[7] is the leading cause of premature death in the US. The NIH considers serum total cholesterol (TC) and cholesterol lipid fractions LDL and HDL as valid biomarkers for the risk of CVD; consequently, interventions that lower LDL cholesterol (LDL-C) and/or increase HDL cholesterol (HDL-C) concentrations are considered beneficial to health.

CVD is a lifestyle-related disease and positive changes in physical activity and diet may result in substantial improvements.

Metabolic syndrome (MetSyn) is a cluster of metabolic conditions that signal risks for coronary heart disease and Type 2 diabetes (1,2). This syndrome is characterized by conditions that include increased central adiposity, higher than normal serum triglycerides (TG) and LDL-C, and low HDL-C, high serum glucose, and high blood pressure. Resistant starches (RS) in the diet may assist in the management of MetSyn by delaying the delivery of glucose as fuel by prolonging absorption, changing fat utilization, and controlling appetite through increased satiety (3). Feeding isolated RS from corn significantly lowers TC, LDL-C, and fasting serum glucose in mildly obese humans (4).

The American Dietetic Association recommends consuming a variety of whole foods rather than supplements alone to obtain the various nutrients and other food components required for good health (5). Whole, cooked dried beans are excellent sources of RS. Additionally, they provide high quality protein, are low in fat, and contain many beneficial substances such as vitamins, minerals, and polyphenols. Studies have shown that beans of selected varieties such as navy beans and chickpeas, in multiple

servings per day, will lower serum lipids (6). Although pinto beans have a similar composition as navy beans and chickpeas, their effects on serum lipids have had limited exploration. Some of the modifying effects of beans on blood lipids might result from their RS content, which when fermented by bacteria in the large intestine produces SCFA that might alter metabolic pathways. One of the major SCFA produced by fermentation is propionate, which has been associated with reduced serum cholesterol (7). Thus, increasing the SCFA produced by fermentation of RS might be an underlying reason for the CVD-protective benefits afforded by the consumption of dry beans.

Although it is commonly assumed that the consumption of dry beans is associated with flatulence and hence the production of volatile products, few experiments have been conducted in humans. Studies in rats report increased butyrate production from dry beans, but because of coprophagia, rats are poor models for lower bowel fermentation experiments. Ruminant nutritionists have long relied on in vitro fermentation assays, where suitable substrates and growth media are inoculated with ruminal liquor (8). Recently, similar studies were used to determine the fermentation aspects of RS in humans, where human fecal preparations were used as the inoculum source (9). These procedures are based on the assumption that the excreted feces contain bacterial populations similar to those found in the lower gut.

The 3 primary objectives of the current human study were to determine whether the consumption of 1/2 cup (130 g) of cooked dried pinto beans per day for 12 wk would: *1*) alter in vitro fecal bacterial fermentation in a manner consistent with benefits to health, primarily toward cancer and CVD; *2*) change the populations of selected bacterial species that are associated with changes in in vitro fermentation when excreted fecal material is used as the inoculum for various RS substrates; and *3*) change serum lipid profiles that are consistent with positive effects on CVD. Because of the prevalence and health implications of MetSyn, a corollary of all objectives was to determine whether any health benefits occurred, or were magnified, in volunteers preconditioned to MetSyn (pre-MetSyn), i.e. volunteers with MetSyn criteria levels higher or lower than normal but not different enough to be considered an advanced state of MetSyn (a summary of the signs of MetSyn can be found in Table 2 of reference 10).

Volunteers and Methods

Volunteer recruitment and selection. All aspects of this study were approved in advance by the Institutional Review Board of the University of North Dakota. Forty men and 40 women aged 18–55 y were recruited locally by newspaper, radio, TV, and Internet advertisements. All volunteers were prescreened for study eligibility, which included age, waist circumference, general health, antibiotic consumption, and willingness to eat beans. Prospective volunteers were informed of all aspects of the study and each signed a written informed consent.

Prior to beginning the study, volunteers received a complete general health screening and blood was drawn after an overnight fast. Measurements included a complete blood count (CellDyne 3500, Abbot Laboratories), thyroid-stimulating hormone, C-reactive protein (Immulite 1000 Analyzer, Diagnostic Products), cholesterol (total, LDL, and HDL) and TG, blood glucose, liver and kidney function (Cobas Mira Analyzer, Roche Diagnostic Systems), blood pressure, a breath sample (for breath methane; see below), body measurements including waist and hip circumferences, and skin fold measurements to calculate body fat content. Blood chemistry methods were those used in a standard clinical laboratory setting. We measured waist circumference on exposed skin in the abdominal area while the subject stood erect and relaxed. Measurements were made with a spring-loaded, inelastic tape placed in a horizontal position at the level of the natural waist, at the lowest part of

the torso (11). Blood pressure and body composition were measured as described by Lukaski et al. (12) and Lohman (13).

Study design. The study design was a 2 × 2 factorial with and without pre-MetSyn and 2 dietary intervention groups (beans or chicken noodle soup). The primary criterion for pre-MetSyn was waist circumference, ≥96.5 cm for men and ≥88.9 cm for women. Waist circumference was <96.5 cm for control men and <88.9 cm for control women. In addition to waist circumference, at least 1 of the following criteria had to be met for pre-MetSyn designation: serum HDL-C <55 mg/dL (1.42 mmol/L); serum TG between 150 and 199 mg/dL (1.69–2.25 mmol/L); fasting blood glucose between 100 and 125 mg/dL (5.6–6.9 mmol/L); or blood pressure between 120/85 and 140/85 mm Hg. Control volunteers had values for these parameters within the normal ranges (entry values for all volunteers can be found in **Table 1**). Healthy volunteers were age and sex matched to pre-MetSyn volunteers. Potential volunteers also were blocked, as much as possible within the experimental design, as producers or nonproducers of breath methane. Medication use for the healthy volunteers was severely restricted, but pre-MetSyn volunteers were allowed to take medication for MetSyn-related disorders (e.g. high blood pressure but not high blood lipids). Smokers were eligible to participate. However, volunteers were asked to refrain from donating whole blood, platelets, or plasma during the study.

The study lasted 16 wk and included a 4-wk equilibration period followed by a 12-wk dietary intervention period. Volunteers lived at home and consumed their own self-selected diets with restrictions that included no beans of any type except those provided by the study, no dietary supplements, no pre- or probiotic foods or supplements, and no prescription or over-the-counter medication to reduce intestinal gases. During the equilibration period, volunteers consumed only their regular diet with the above restrictions. During the 12-wk dietary intervention period, volunteers were asked to add 1 of 4 different bean or soup entrées per day to their normal diets. The entrées contained either cooked pinto beans or chicken soup prepared at the Grand Forks Human Nutrition Research Center (**Supplemental Table 1**). The bean entrées were standard servings of cooked pinto beans [130 grams (1/2 cup); canned by Bush Brothers and generously donated by Archer Daniels Midland]. Each soup entrée was isonitrogenous and isocaloric as near as possible with respect to each bean entrée. At the end of both the equilibration and intervention periods, each volunteer was asked to keep a dietary record of the food consumed over a 3-d period. This period included 2 weekdays and 1 weekend day, consecutively. Each record was reviewed for accuracy by a registered dietitian with the volunteer present and was later analyzed by computer for macronutrient intake (see below).

Study restrictions. Clinical screening results were reviewed by a physician to determine whether the applicants met the basic qualifications or whether they were ineligible because of a possible need for medical attention. If the latter occurred at any point in the study, clinical data were given to the volunteer and they were encouraged to see their personal physician. Because antibiotics will kill or dramatically change gut microflora populations, volunteers who had taken antibiotics within 6 mo of the beginning of the study were ineligible and volunteers who began taking antibiotics during the study were asked to withdraw.

Sample collection. Samples were collected from volunteers at the end of the equilibrium period and at the end of the intervention period after an overnight fast. Sample analyses included blood lipid profile (TC, lipoprotein fractions, and total TG), hematology (red and white cell count, platelets, hematocrit, and red cell size distribution), C-reactive protein, and blood acetate. In addition, each volunteer collected a fecal sample at home from a single bowel movement and delivered it to the laboratory within 4 h of collection. Part of the sample was used immediately for the determination of SCFA production by in vitro fermentation. The remainder was frozen at −80°C and used later for bacterial speciation (see descriptions below).

Fecal in vitro fermentation and SCFA analysis. In a clean clinical laboratory environment, the 4-h fresh fecal sample was thoroughly mixed in an airtight plastic bag and a 100-μL aliquot was removed from

TABLE 1 Characteristics of volunteers upon entry into the study[1,2]

| | Women | | | | Men | | | |
| | Control | | Pre-MetSyn[3] | | Control | | Pre-MetSyn | |
	Soup	Beans	Soup	Beans	Soup	Beans	Soup	Beans
Age, *y*	42.2 ± 8.7	43.1 ± 7.1	44.3 ± 12.1	45.8 ± 5.5	30.7 ± 12.3	33.7 ± 12.3	40.4 ± 11.6	39.1 ± 10.0
Weight, *kg*	59.9 ± 4.4	62.9 ± 10.3	90.6 ± 13.1	89.2 ± 14.0	82.4 ± 9.8	76.3 ± 8.1	99.6 ± 10.1	102.7 ± 11.0
Fat-free weight, *kg*	43.4 ± 3.0	45.8 ± 5.8	61.4 ± 9.2	61.9 ± 8.6	65.1 ± 6.2	62.3 ± 5.4	76.8 ± 8.7	79.0 ± 8.4
Fat weight, *kg*	15.3 ± 3.1	16.9 ± 5.6	27.3 ± 7.0	29.9 ± 8.1	14.2 ± 3.7	11.9 ± 4.4	20.3 ± 2.1	21.9 ± 5.0
Waist circumference,[4] *cm*	77.7 ± 4.6	77.5 ± 6.1	103.2 ± 11.9	105.3 ± 14.5	88.3 ± 6.3	83.3 ± 5.5	106.8 ± 8.0	108.9 ± 10.2
Waist:hip ratio	0.83 ± 0.05	0.83 ± 0.06	0.88 ± 0.06	0.89 ± 0.05	0.92 ± 0.04	0.91 ± 0.05	0.99 ± 0.06	0.97 ± 0.07
BMI, *kg/m²*	22.7 ± 1.8	23.3 ± 3.2	33.1 ± 3.6	34.5 ± 5.6	26.0 ± 3.0	23.9 ± 2.6	31.9 ± 3.4	31.8 ± 2.0
BP systolic, *mm Hg*	104 ± 5	106 ± 8	123 ± 8	120 ± 8	114 ± 6	113 ± 3	126 ± 10	122 ± 11
BP diastolic, *mm Hg*	70 ± 7	71 ± 26	80 ± 5	80 ± 8	69 ± 5	71 ± 5	80 ± 8	78 ± 6
Serum TC,[5] *g/dL*	179 ± 47	177 ± 29	196 ± 30	198 ± 15	158 ± 37	179 ± 28	199 ± 35	192 ± 20
Serum TG, *g/dL*	64.3 ± 17.4	76.0 ± 30.7	112.7 ± 40.2	110.1 ± 47.5	83.5 ± 23.4	62.9 ± 22.8	112.2 ± 44.9	119.9 ± 56.1
Serum HDL-C, *g/dL*	62.3 ± 11.4	67.8 ± 18.3	58.4 ± 11.3	59.9 ± 13.2	52.5 ± 7.6	58.7 ± 11.9	53.8 ± 10.4	47.7 ± 5.6
Serum glucose, *g/dL*	84.7 ± 10.1	80.1 ± 8.3	92.8 ± 18.4	87.6 ± 15.2	86.7 ± 6.5	78.4 ± 8.1	98.8 ± 11.2	92.2 ± 7.9

[1] Values are mean ± SD, *n* = 10.

[2] Volunteers were not consuming prescribed soup or beans at this point. These notations were used to indicate group assignments only.

[3] pre-MetSyn; defined as MetSyn criteria levels higher or lower than normal, but not high or low enough to be considered an advanced state of MetSyn (10,21).

[4] Primary selection criterion.

[5] The factors to convert serum parameters to SI units: cholesterol and HDL-C, 1 mg/dL = 0.02586 mmol/L; TG, 1 mg/dL = 0.0113 mmol/L; glucose, 1 g/dL = 0.0555 mmol/L.

the center of the mass and used as the inoculum for an in vitro fermentation study. The in vitro fermentation technique was adapted from methods of Weaver et al. (9) used for assessment of human lower bowel fermentation and from methods for ruminant fermentation by Nelson and Finley (8). Briefly, fresh feces were mixed with a buffer solution and centrifuged to remove debris and the supernatant was used as the inoculum. Anaerobic fermentation vessels with septa were loaded with 100 mg of substrate (dried bean powder, cornstarch, inulin, oat bran, or no substrate controls) and 5 mL of the supernatant from the fecal suspension. Vessels were then purged with N_2/CO_2 and placed on a 37°C shaker for 24 h. Samples were allowed to vent during the incubation. SCFA analysis of each sample was conducted in triplicate on acidified, filtered solutions using GC conditions described by Weaver et al. (9,14).

Fecal bacterial speciation. Populations of fecal bacterial species that are markers of specific metabolic routes were quantified from fresh-frozen stool samples using rRNA-directed, species-specific probes (15). Bacterial DNA was isolated using the QIAamp DNA Stool Mini kit (Qiagen) (bacterial species and primer sequences are listed in **Supplemental Table 2** and procedures for these assays are outlined in **Supplemental Table 3**).

Dietary records analyses. Individual items in the 3-d food records were analyzed for nutrient content by using a computerized nutrient database called Grand Forks Research Analysis of Nutrient Data. It is based in part on the online version of the USDA Nutrient Database for Standard Reference, release 19 (16), and on actual data generated by food analyses in our laboratory. An average daily intake was computed for energy, protein, carbohydrate, lipid, and fiber.

Other measures. Breath samples for measurements of methane were obtained by using an AlveoSampler mouthpiece assembly with collection bag (QuinTron). Methane was analyzed by methods outlined by Behall et al. (17) and Weaver et al. (18).

Statistical analysis. The study size (20 per group) was based on a power analysis of the results of a previous similar clinical trial that measured SCFA in human fecal fermentation (our unpublished data) and blood lipid data based on the work of Poppitt et al. (19). A total of 86 volunteers began the study, but 6 women and 1 man did not finish for

various reasons. For some variables, a change score was calculated by subtracting the baseline value (equilibrium) from the value obtained at the end (intervention) of the study. Two-way ANOVA (SAS) was used to test for differences in the change scores between categories (controls or pre-MetSyn) and diets (beans or soup) or their interaction. For others, such as substrate fermentation of fecal bacteria, we conducted a 2-way ANOVA with categories and diet as the variables. Significance was set at $P \leq 0.05$. No interactions were found; thus, no post hoc test were conducted.

Results

The primary selection characteristic for the volunteers was waist circumference, ≥96.5 cm for men and ≥88.9 cm for women. BMI, blood pressure, and blood lipid concentrations were higher, for the most part, than the controls (Table 1), but none of the other characteristics except waist circumference and waist: hip ratio was at a level that would classify them as having advanced MetSyn (1,10,20,21).

As a result of dietary intervention of 1/2 cup of bean or chicken soup per day for 12 wk, volunteers consumed more ($P <$ 0.001) fiber during the intervention period compared with the intake during the equilibration period, but control and pre-MetSyn groups did not differ (**Table 2**). Pre-MetSyn volunteers consumed more protein ($P <$ 0.025) than the controls during both periods of treatment. Volunteers with pre-MetSyn consumed more lipid ($P <$ 0.005) than controls; however, all volunteers consumed less lipid ($P <$ 0.001) during the intervention period compared with that consumed during the equilibration period (Table 2).

Objective 1 was to determine the effects of bean consumption on in vitro fecal bacterial fermentation and the production of SCFA. Initial studies established that a fermentation period of 24 h did not exhaust the substrate. Others have reported that SCFA production is dependent upon the fermentation substrate (9); thus, the present study used multiple substrates and, as expected, the substrate had a large effect on SCFA production. When the findings were expressed as a difference between the

TABLE 2 An estimate of the mean daily consumption of energy, protein, carbohydrate, lipid, and fiber during the equilibration and intervention periods[1]

| | Equilibration[2] | | | | Intervention | | | |
| | Control | | Pre-MetSyn[3] | | Control | | Pre-MetSyn | |
Nutrients	Soup	Beans	Soup	Beans	Soup	Beans	Soup	Beans
Energy,[4] kcal	2157 ± 94	2318 ± 125	2380 ± 115	2395 ± 102	2242 ± 157	2048 ± 113	2262 ± 116	2332 ± 115
Protein,[5] g	82 ± 5	83 ± 4	97 ± 5	89 ± 5	87 ± 6	76 ± 5	94 ± 5	90 ± 5
Carbohydrate, g	253 ± 18	285 ± 19	244 ± 13	282 ± 16	275 ± 18	260 ± 16	250 ± 13	278 ± 17
Lipid,[6] g	84 ± 5	89 ± 6	108 ± 7	100 ± 5	83 ± 7	72 ± 5	95 ± 7	92 ± 6
Fiber,[7] g	16 ± 2	16 ± 1	16 ± 1	15 ± 1	19 ± 2	20 ± 1	20 ± 2	19 ± 1

[1] Values are means ± SEM, $n = 19$–20, and were derived using the USDA Nutrient Database for Standard Reference, release 19, plus direct in-house analyses of food items (16).
[2] During the equilibration period, volunteers did not consume prescribed soup or beans. These notations were used to indicate group assignments only.
[3] pre-MetSyn; defined as MetSyn criteria levels higher or lower than normal, but not high or low enough to be considered an advanced state of MetSyn (10,21).
[4] 1 kcal = 4.186 kJ.
[5] For protein consumption, pre-MetSyn > control, $P < 0.025$.
[6] For lipid consumption, pre-MetSyn > control, $P < 0.005$; intervention < equilibration, $P < 0.001$.
[7] For fiber consumption, intervention > equilibration, $P < 0.001$.

equilibration and the interventions periods, propionic acid ($P < 0.02$) and total fatty acid ($P < 0.05$) productions (mmol/kg dry feces) were higher in volunteers who consumed beans compared with those who consumed soup (data not shown), although only when bean flour was used as the substrate. There was no effect of pre-MetSyn. When we collected data at the end of the treatment period and analyzed them as a 2 × 2 factorial, there was no effect of bean consumption on total SCFA production, regardless of substrate (**Fig. 1A**). Total SCFA was higher in pre-MetSyn volunteers than controls only when cornstarch ($P < 0.02$) and inulin ($P < 0.003$) were used as substrates. Acetic acid (Fig. 1B) concentrations posttreatment were not significantly affected by bean consumption. Propionic acid production from cornstarch and oat bran was higher ($P < 0.05$) in those who were pre-MetSyn than controls (Fig. 1C). Butyric acid production from inulin was higher ($P < 0.002$) in pre-MetSyn than controls (Fig. 1D) but was not affected by bean consumption. Butyric acid production from cornstarch was lower ($P < 0.02$) in the feces of those who consumed beans than in those who consumed soup.

Volunteers who consumed beans had lower production of isovaleric acid from cornstarch ($P < 0.05$) and oat bran ($P < 0.03$) than those who ate soup (**Fig. 2A**). Isobutyric acid production from cornstarch ($P < 0.002$), inulin ($P < 0.03$), and oat bran ($P < 0.02$) was lower in volunteers who consumed beans than in those who consumed soup (Fig. 2B). Isobutyric acid was

higher ($P < 0.03$) in the pre-MetSyn group than controls. Acetate, propionate, and butyrate are normally produced in approximate molar ratios of 60:20:20, respectively (22,23). In this experiment, the ratios were 66:18:16 across all substrates. However, when the substrate inulin was considered alone, the ratios changed to 58:22:20 (**Fig. 3**).

Objective 2 was to assess changes in bacterial populations caused by consuming beans. Bean consumption had no significant effect on most of the bacteria populations except for *Eubacterium limosum* (**Fig. 4; Supplemental Fig. 1**). The population of *E. limosum* between pre-and posttreatment was lower ($P < 0.009$) in volunteers consuming beans compared with the change in those consuming soup in both the control and pre-MetSyn groups. The population of *Peptostreptococcus productus* was higher ($P < 0.01$) in the feces of the pre-MetSyn group compared with controls but was not affected by bean consumption.

Objective 3 was to determine whether bean consumption changed serum lipid profiles in a manner consistent with positive effects on the risk of CVD. The changes in TC ($P < 0.014$) (**Fig. 5A**) and HDL-C ($P < 0.05$) (Fig. 5B) between pre- and post-treatment were lower in those volunteers who consumed beans than in those who consumed soup in all volunteers (pre-MetSyn or controls). This amounted to an ~8% reduction in TC in the control group and a 4% reduction in the pre-MetSyn group.

FIGURE 1 The consumption of 1/2 cup (130 g) of cooked dried beans pinto daily for 12 wk with or without pre-MetSyn affected posttreatment production of SCFA in feces of humans. Values are µmol/g dry weight of feces. Means ± SEM, $n = 19$–20. Within cornstarch substrate where pre-MetSyn > controls, total fatty acids = $P < 0.02$ and propionic acid = $P < 0.05$; and beans < soup, butyric acid = $P < 0.02$. Within the inulin substrate where pre-MetSyn > controls, total fatty acids = $P < 0.003$ and butyric acid = $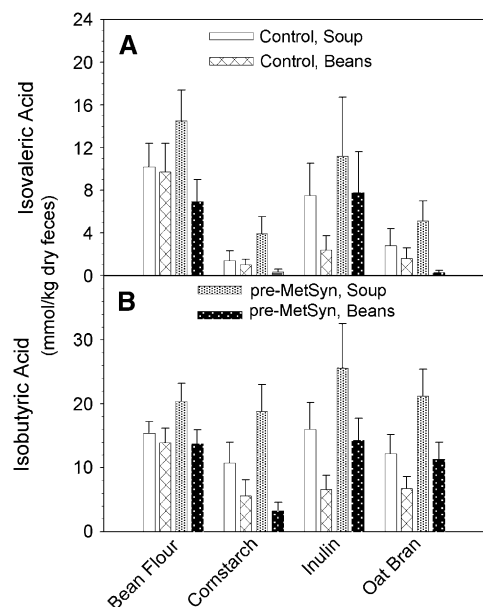P < 0.002$. Within the oat bran substrate where pre-MetSyn > controls, propionic acid = $P < 0.04$ and butyric acid = $P < 0.05$.
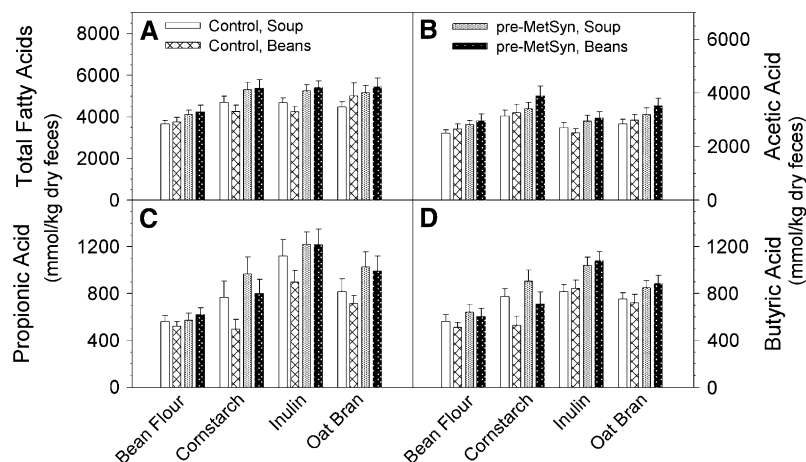
FIGURE 2 Effect of consumption of 1/2 cup (130 g) of cooked dried beans pinto daily for 12 wk with or without pre-MetSyn affected the posttreatment production of isovaleric and isobutyric acids from various substrates in feces of humans. Values are μmol/g dry weight of feces. Means ± SEM, $n = 19$–20. Within cornstarch substrate where beans < soup, isovaleric acid = $P < 0.05$ and isobutyric acid = $P < 0.002$. Within inulin substrate where beans < soup, isobutyric acid = $P < 0.03$. Within oat bran substrate where beans < soup, isobutyric acid = $P < 0.02$ and pre-MetSyn > control, isobutyric acid = $P < 0.03$.

There was an 8% reduction in HDL-C in the control group and a 4% reduction in HDL-C for the pre-MetSyn group. Bean consumption lowered serum LDL-C ($P < 0.05$) ~7%, but there was no effect of pre-MetSyn compared with the controls (Fig. 5C). Bean consumption did not alter serum TG (Fig. 5D), VLDL cholesterol, or glucose (the latter 2 not shown). This part of the study demonstrates that a consistent intake of a normal serving (1/2 cup; 130 g) of pinto beans per day in combination with a regular diet could effectively lower blood lipids in control individuals as well as those predisposed to MetSyn.

Discussion

Diet is a primary risk factor for the development of chronic disease in Western cultures. CVD, the leading killer, is especially affected by diets that are energy dense and contribute to obesity. Cancer is the 2nd leading cause of premature death in the US and Doll and Peto (24) have estimated that nearly 40% of all cancers are diet-related. Thus, dietary changes that can reduce the risk of these conditions have important public health implications. This study has demonstrated that within the context of a self-selected Western diet, substitution of a modest portion of the diet with pinto beans each day compared with an isocaloric and isonitrogenous soup results in a favorable shift in lipid profiles. Further, our study employed rigorous screening methods to recruit healthy and mildly MetSyn individuals and to balance these subgroups between those who consumed beans and those who consumed soup as a control. We have demonstrated that the addition of beans to the diet could be beneficial to healthy individuals as well as those preconditioned for MetSyn by
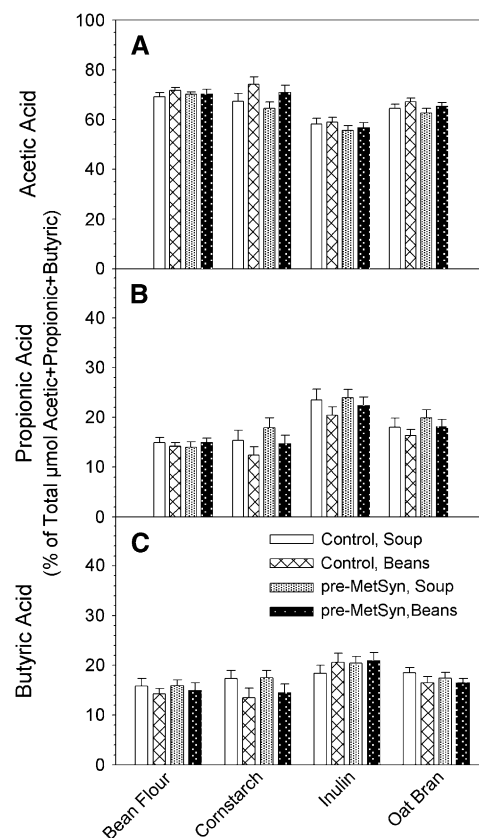

FIGURE 3 The consumption of 1/2 cup (130 g) of cooked dried pinto beans daily for 12 wk with or without pre-MetSyn affected the ratios of acetic, propionic, and butyric acids produced during fermentation of various substrates in the feces of human volunteers. Values are μmol/g dry weight of feces. Means ± SEM, $n = 19$–20.

lowering serum TC and LDL-C. The treatment differences were of such magnitude that significance was found to be both statistical and physiological. However, we have no explanation for some findings. These included lower concentrations of HDL-C and no change in TG in the serum of those who consumed beans.
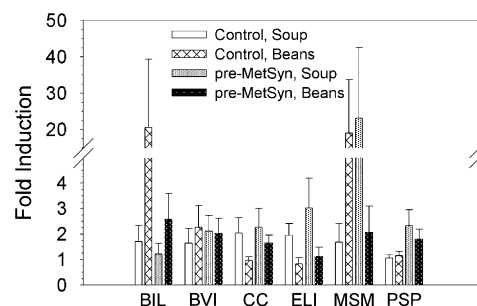

FIGURE 4 The consumption of 1/2 cup (130 g) of cooked dried beans daily for 12 wk with or without pre-MetSyn lowered ($P < 0.009$) *E. limosum* bacterial populations in the feces of human volunteers. *P. productus* population was higher ($P < 0.01$) in pre-MetSyn than controls. Values are means ± SEM, $n = 17$–20. Two values exceeding ± 2 SD of the mean were removed as outliers. BIL, *Bifidobacterium longum*; BV, *Bacteroides vulgatus*; CC, *Clostridium clostridiiforme*; ELI, *E. limosum*; MSM, *Methanobrevibacter smithii*; and PSP, *Peptostreptococcus productus*.

FIGURE 5 Volunteers who consumed of 1/2 cup (130 g) of cooked dried beans daily for 12 wk with or without pre-MetSyn had reduced serum TC ($P < 0.014$), HDL-C ($P < 0.05$), and LDL-C ($P < 0.05$) but not TG. Values are means ± SEM, $n = 19$–20, and are expressed as the difference between those found at the end of the equilibration period and at the end of the intervention period. The factors to convert parameters to SI units: cholesterol, HDL-C, and LDL-C, 1 mg/dL = 0.02586 mmol/L; TG, 1 mg/dL = 0.0113 mmol/L.
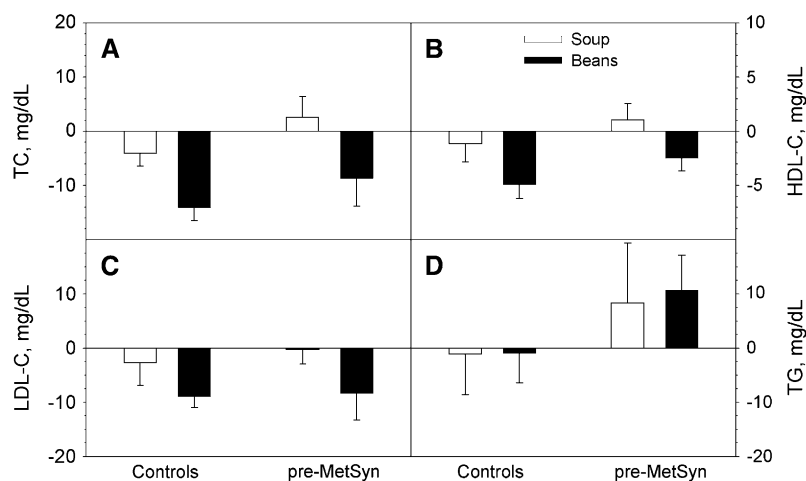
Taken alone, these data are intriguing, but put into the context of many previous reports of the lipid-modulating properties of dry bean consumption they show a pattern of substantial health benefits. Although many reviews of this field have not clearly delineated the benefits between dietary fiber and those of dry beans (the category "beans" often includes studies conducted with soy beans or other soy products), there are still substantial numbers of studies showing that dry beans alone have a clear lipid-modulating effect (25–27). Clinical trials are usually predicated on clear epidemiologic evidence, but there is only 1 reported epidemiologic trial specific to legumes. This was a follow-up to NHANES I in which men and women who consumed legumes ≥4 times per week decreased their risks of coronary heart disease (22%) and CVD (11%) compared with those consuming <1 serving per week (27). Clinical trial data are more common, which include metabolic studies and studies with free-living volunteers; trials also have used both healthy volunteers and those with health-compromising conditions. Although a few studies with hyperlipidemic men reported no effects of bean consumption (28–30), most have shown clear benefits of beans. Anderson et al. (31) fed hyperlipidemic men 120–162 g/d pinto beans and reported an average reduction in serum cholesterol of 10.4%. Another report with hypercholesterolemic men found that beans were as effective as oat bran in reducing serum cholesterol (32). Mixed legumes substituted for an equal amount of calories, protein, carbohydrate, and fat in the diets of normal men resulted in ~10% lower LDL-C than those consuming diets with no legumes (33). Fruhbeck et al. (34) reported that normal or borderline high-cholesterol men had decreased LDL-C, VLDL cholesterol, and TC but higher HDL-C following consumption of bean flour. Shutler et al. (35) fed normal young males 450 g/d of canned baked beans and showed a 10% drop in cholesterol compared with those receiving no beans.

Thus, a clear implication to public health is emerging and incentives should be developed to encourage the consumption of dry beans. Although overall statistics show a rise in bean consumption over the past 4 decades to a high of ~47 g/d in 2003, overall bean consumption has trended downward for the past 10 y (36). In addition, when considered by specific groups, the data give a different picture. Hispanics of Mexican descent in the US consume ~186 g/d of beans, whereas U.S. Caucasians consume only an average of 33 g/d. Further, males are the predominant consumer of beans, whereas the Caucasian female consumes the lowest amount of all groups (37).

Our original hypothesis, which is similar to that of many other investigators, was that the fermentation of the fiber from beans would cause a change in the resident gut bacterial populations and the evolution of specific SCFA and this would be the primary reason for any observed health benefits of beans. However, there were no clear trends in the change of bacterial populations and the only significant change in fermentation patterns from baseline was a greater production of propionate and total fatty acids when bean flour was the substrate in volunteers consuming beans compared with those consuming soup. Although the change in propionate was modest, it may be physiologically significant, because the cholesterol-lowering properties of propionate are well characterized (7,38–41). Propionate, unlike acetate, is not a substrate for lipogenesis and increased propionate production has been reported to inhibit fatty acid synthesis (40,42).

Although the changes in individual fatty acids between subjects who consumed beans and those who consumed soup were intriguing, perhaps a more notable finding of this study was the lack of more and greater changes in fermentation. Thus, we conclude that perhaps the dietary fiber/fermentation hypothesis has been overstated and many of the real health benefits of eating beans are from components of beans not associated with fermentation. Support for this theory comes from a study showing significant reductions in cholesterol following the consumption of bean extracts (43). Although the composition of the extracts used was not given in detail, such extracts available as dietary supplements are generally water or alcohol soluble components that are condensed and dried. Although some fibers are retained in the extracts, the percentage would be much less than that in the whole bean. Other bioactive components, notably phenolic compounds (including flavanoids), would most likely predominate, suggesting that the health benefits are brought about by metabolic routes unrelated to fermentation. Many clinical, animal, and in vitro studies have reported the lipid-modifying effects of dietary phenolics from various preparations and extracts made from beans (44–48).

In conclusion, this study adds to a growing and convincing body of evidence that adding dry beans to the diet in quantities of at least 100 g/d changes lipid profiles in a manner associated with decreased risk of CVD. Moreover, individuals with signs and symptoms of MetSyn seem to benefit as much if not more than normal individuals and these individuals are at greatest risk for CVD. Although the health benefits of beans are becoming more apparent, the mechanistic basis for this action is not clear.

In this study, changes in gut fermentation patterns were not sufficient to explain the changes in lipids, suggesting that future studies should expand the mechanistic hypothesis and examine other bioactive components of beans.

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